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Pulmonary and Cutaneous Gas Exchange in the Green Frog, *Rana clamitans*[1]

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(Text-figures 1-4)

THE Amphibia have developed several respiratory mechanisms which occur in various combinations: branchial, buccopharyngeal, pulmonary and cutaneous. Krogh (1904) performed the first quantitative study of pulmonary and cutaneous respiration in amphibians on the European frogs *Rana esculenta* and *R. temporaria*. Pulmonary respiration was separated from cutaneous respiration by cannulating the lungs. The cannula was then connected through a stopper in the respiration chamber to a system of circulating air separate from the one in which cutaneous respiration was taking place. Air was pumped mechanically into the lungs at regular intervals. Similar techniques were used by Dolk & Postma (1927) on *R. temporaria*. These works showed that cutaneous oxygen uptake remained relatively constant throughout the year. Pulmonary oxygen uptake was greatest in the spring and minimal during the fall and winter. Pulmonary and cutaneous carbon dioxide release showed the pattern of high release in the spring and low in the fall and winter with the greatest percentage of release being through the skin at all times. Two experimental errors were introduced into the above works. First, the animals were not acclimated to constant temperature and photoperiod and second, artificially pumping air in and out of the lungs did not allow the frogs to carry on their normal breathing movements (Scholten, 1942; Cherian, 1958).

In the United States, Whitford & Hutchison (1963, 1965, MS) improved the methods of measuring gas exchange and acclimating the animals in their work on several species of salamanders. The present study represents the first application of these improved methods to anuran respiration. A comparison of respiratory mechanisms in frogs and salamanders will give further clues to the evolution of respiratory mechanisms in the Amphibia and the relation of these mechanisms to the habitats of the animals.

Materials and Methods

The animals used for this study were collected in Washington County, Rhode Island, during 1963. They were acclimated at a constant temperature and photoperiod for at least two weeks before they were used in an experiment. Acclimation temperatures were 5°, 15° and 25°C. The photoperiods used were 8 and 16 hours (8L16D = 8 hours of light, 16 hours of dark, and 16L8D = 16 hours of light, 8 hours of dark, respectively).

Respiration was measured in a closed system respirometer consisting of four equal-volume chambers. The animal was placed in one of the front chambers and a mask of tygon tubing, which covered the front part of the head without obstructing the nares, was connected to the other front chamber through an opening between the two chambers. Each of the front chambers was connected through a manometer to a rear chamber which acted as a thermobarometer. The method is described in more detail by Whitford & Hutchison (1963) and differs from their description only in that the carbon dioxide was absorbed with sodium hydroxide rather than barium hydroxide. Sodium hydroxide forms a soluble carbonate, thus eliminating the necessity

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of regularly breaking up the insoluble film of barium carbonate formed when barium hydroxide is used.

Respiration measurements were determined for five-hour periods on animals acclimated at 15° and 25°C, and for 26-hour periods on animals acclimated at 5°C. The 5° animals were run for a full day to determine if any daily fluctuations occurred in gas exchange. The decision to do this was made after the 15° and 25° experiments had been completed. The light and dark periods at the time of the experiment were the same as those during the acclimation period. A red darkroom bulb was used to make the instrument readings during the dark hours.

Results

Pulmonary and Cutaneous Gas Exchange

All values of gas exchange are given in microliters per gram per hour (μl/g/hr). Pulmonary oxygen consumption increased almost linearly from 3.20 at 5°C to 60.11 at 25°C for 8L acclimated animals and linearly from 8.91 at 5°C to 60.06 at 25°C for 16L animals. Cutaneous oxygen uptake increased slightly from 14.21 to 22.99 for 8L animals and from 11.73 to 21.32 for 16L animals (Table 1). The ratio of pulmonary to cutaneous uptake increased with temperature (Text-fig. 1). A significant difference in the ratio between 8L and 16L animals is apparent at 5°C ($t = 2.86$, $p < 0.025$). No significant difference was found at 15° or 25°C.

Pulmonary carbon dioxide release increased from 1.92 at 5°C to 15.59 at 25°C for 8L animals and from 1.96 at 5°C to 17.07 at 25°C for 16L animals. Cutaneous carbon dioxide release increased from 13.02 to 59.25 for 8L animals and from 16.12 to 55.42 for 16L animals (Table 1). The ratio of pulmonary to cutaneous carbon dioxide release increased only slightly from 5°C to 25°C (Text-fig. 2). Over 80% of the carbon dioxide released at all temperatures was through the skin.

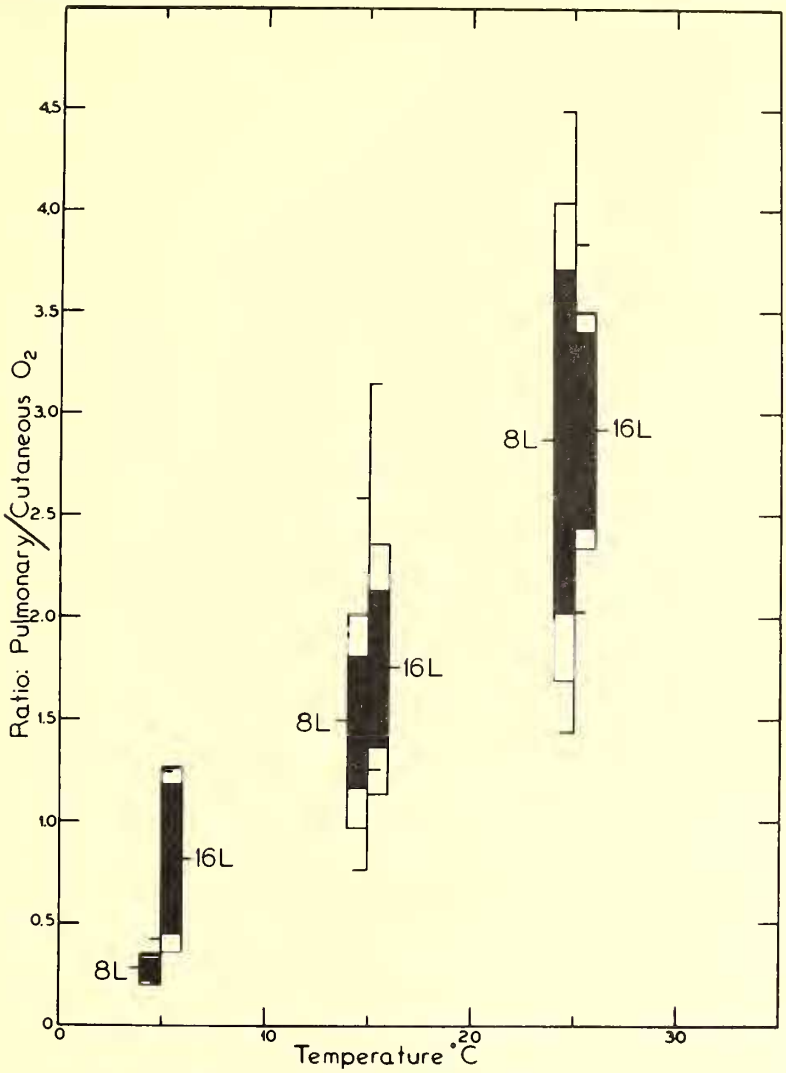
Text-fig. 3 shows the relationship between pulmonary and cutaneous gas exchange in 16L animals. A plot of the data for 8L animals would show the same relationships.

Relationship of Body Weight to Oxygen Uptake

Oxygen uptake is plotted as a function of weight in Text-fig. 4. The data are plotted at three temperatures with the 8L and 16L data combined at each temperature. A regression of metabolic rate on body weight was determined at each temperature and the lines plotted on the same figure. The general equation is M (metabolism) = kW (body weight)^n or log M = log K + n log W. Log K represents the y-intercept

Table 1—Values for Pulmonary and Cutaneous Gas Exchange in Microliters Per Gram Per Hour (ul/g/hr)

Temp.	Photoperiod	N-hrs	N-anim	Range	Mean	SD	SE	Range	Mean	SD	SE
				Oxygen Pulmonary				Cutaneous			
25°C	8L	40	8	24.19-131.19	60.11	23.61	3.73	5.04-59.29	22.99	11.27	1.78
25°C	16L	30	6	37.38-120.21	60.06	19.42	3.54	8.41-36.63	21.32	6.59	1.20
15°C	8L	50	10	3.17-66.37	27.90	12.69	1.79	4.71-46.13	19.41	10.34	1.46
15°C	16L	50	10	5.56-84.88	32.12	16.25	2.30	2.78-47.30	19.50	9.58	1.35
5°C	8L	156	6	0-26.92	3.20	4.73	0.12	4.66-37.25	14.21	5.76	0.15
5°C	16L	156	6	0-58.20	8.91	6.70	0.17	2.95-44.26	11.73	6.27	0.16
				Carbon Dioxide Pulmonary				Cutaneous			
25°C	8L	40	8	8.98-26.63	15.59	5.53	1.96	46.59-71.30	59.25	8.72	3.08
25°C	16L	30	6	12.62-20.76	17.07	3.16	1.29	47.73-64.22	55.42	6.42	2.62
15°C	8L	50	10	1.59-14.23	8.24	4.96	1.57	24.52-39.90	29.57	4.68	1.48
15°C	16L	50	10	2.38-15.34	7.81	4.48	1.42	23.52-35.27	30.19	3.09	0.98
5°C	8L	156	6	0.53-2.55	1.92	0.75	0.31	10.46-15.32	13.02	1.68	0.69
5°C	16L	156	6	0.55-3.53	1.96	1.09	0.45	13.61-18.35	16.12	1.98	0.81



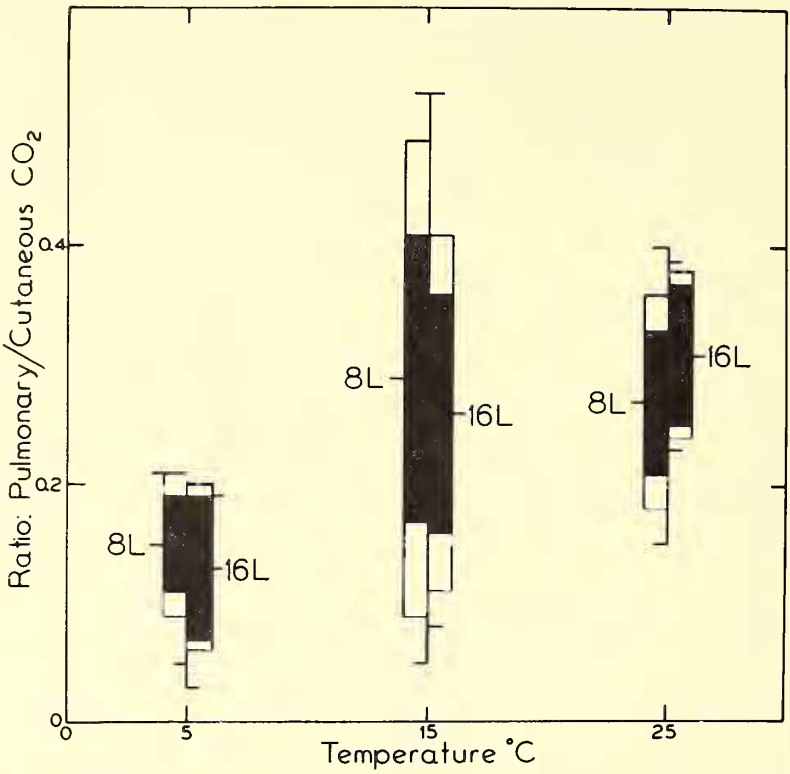
TEXT-FIG. 1. Ratio of pulmonary to cutaneous oxygen uptake at the temperatures and photoperiods indicated. The short vertical line pointing to the photoperiod represents the mean of the sample. One black and one white rectangle combined on one side of the mean represents one standard deviation, and one black rectangle on one side of the mean represents two standard errors. The long vertical line represents the range of the sample, with short horizontal lines delimiting the extent of the range. Short lines pointing to the right delimit 16L ranges and short lines pointing to the left delimit 8L ranges. If there is no overlap between the black rectangles of two sets of data, the difference between the means may be considered statistically significant (Hubbs & Hubbs, 1953).

and n the slope when the data is plotted logarithmically, the equation of the line at 25°C was log M = 0.203 + 0.799 log W; at 15°C, log M = —0.333 + 1.016 log W; at 5°C, log M = —0.372 + 0.753 log W.

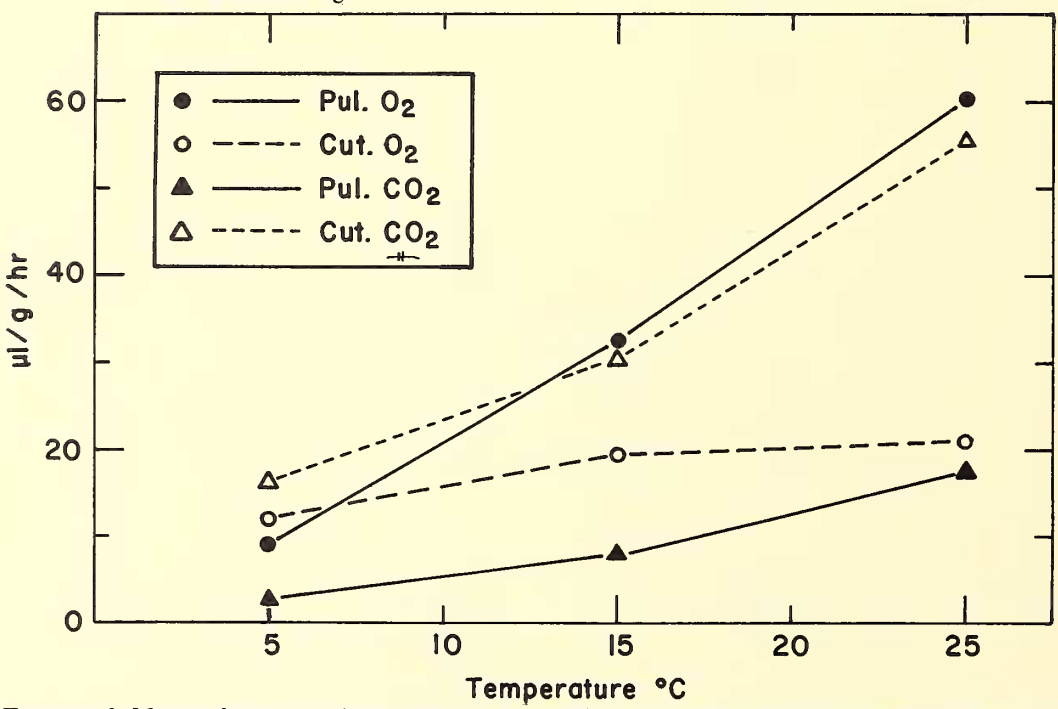
DISCUSSION

In *Rana clamitans* the additional oxygen requirements at higher temperatures are supplied by an increase in the rate of pulmonary respiration. Cutaneous uptake is passive and, therefore, can not supply the additional oxygen needs. The skin, however, is an important respiratory organ as over 80% of carbon dioxide release occurs through the skin at all temperatures.

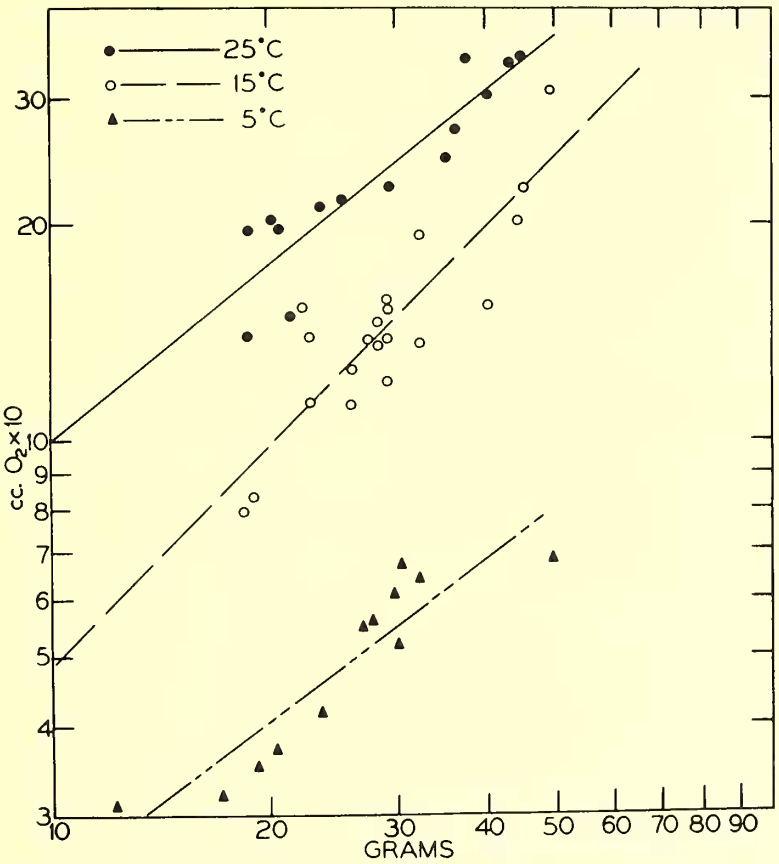
Photoperiod has been shown to effect such



TEXT-FIG. 2. Ratio of pulmonary to cutaneous carbon dioxide release at the temperatures and photoperiods indicated. The method of presentation is the same as in Text-fig. 1.



TEXT-FIG. 3. Mean pulmonary and cutaneous oxygen uptake and carbon dioxide release for 16L animals at the temperatures indicated. Each point represents the mean value for all animals at that temperature.



TEXT-FIG. 4. Relation of oxygen uptake per hour with weight. Each point represents the oxygen uptake and weight of a single animal. The regression lines were obtained by the method of least squares.

phenomena as spring migration and reproductive cycles in birds, breeding cycles of mammals and fish and disapause in insects (Withrow, 1959). Hutchison (1961) found seasonal differences in the critical thermal maximum (CTM) of the newt, *Notophthalmus viridescens*. Hutchison suggested that photoperiod is responsible for the observed seasonal variation in CTM and that it is likely that the animals react to a changing ratio of hours of light to hours of dark rather than to the total number of daylight hours.

Krogh (1904) and Dolk & Postma (1927) found seasonal variation in the pattern of gas exchange in *Rana temporaria*. Fromm & Johnson (1955) noted a similar pattern in *Rana pipiens*. Whitford & Hutchison (1965) found that the spotted salamander, *Ambystoma maculatum* at 15°C had a significantly higher rate of oxygen consumption when acclimated to a 16L photoperiod than when acclimated to a 8L photoperiod. This pattern could easily be due to the seasonal changes in photoperiod.

Brown *et al.* (1955) reported on the occurrence in animals and plants of daily rhythms which persist under conditions of constant darkness and temperature. Many animals maintain their 24-hour cycle, even when phases of the cycle have been experimentally shifted from their normal day-night synchronization to opposite times of light and dark.

Photoperiod significantly affected oxygen uptake only at 5°C in *Rana clamitans* in this study. This does not rule out the possibility that photoperiod has an effect on seasonal oxygen consumption in this animal. The gas exchange of the animals at 5°C was measured over a period of 26 hours, while the animals acclimated at 15° and 25°C were used only for five-hour periods from 1000 or 1100 hours to 1500 or 1600 hours (EST). If any daily rhythmicity exists in their respiratory pattern, then the comparison of measurements taken at the same time of day for 8L and 16L acclimated animals might hide the effect of photoperiod; *i. e.*, metabolism could vary over

a 24-hour period but might be the same in any small segment of time. It would be necessary, therefore, to measure the metabolism at 15° and 25°C over a 24-hour period to see if photoperiod has an effect at these temperatures.

The effect of photoperiod may not be the same at all temperatures. Hutchison & Kosh (1965) studied the effect of photoperiod on the CTM of painted turtles, *Chrysemys picta,* acclimated to 10°, 20° and 30°C. Animals acclimated under 16L had a higher CTM than those acclimated under 8L at all acclimation temperatures. However, the difference between the 8L and 16L animals decreased with increasing acclimation temperature.

The ability of an animal to react to a change in photoperiod would be a definite advantage for the animal. An animal responding to an increasing photoperiod in early spring by raising its metabolic rate would be preadapting itself to the coming warmer temperatures which further increase the metabolism of the animal. Thus, an animal's physiological responses could become preadapted to an increase in temperature before the increase actually occurred.

The value of n in the equation $M = kW^n$ (see explanation of symbols under results) has been shown to be approximately 0.75 for unicellular organisms, plants, poikilotherms and homeotherms (Hemmingsen, 1960). Tashian & Ray (1957) compared oxygen consumption in tropical frogs with consumption in temperate and boreal species. The tropical anurans (*Hyla maxima, H. crepitans, Leptodactylus typhonius, Eupemphix pustulosus, Prostherapis trinitatis*) had an n of 0.83 at 25°C and of 0.86 at 10°C. The temperate and boreal frogs (*Rana sylvatica, H. crucifer, R. clamitans*) had an n of 0.70 at 24°C and 0.71 at 14°C. Cherian (1962) found $n = 0.925$ for *Rana hexadactyla* at 29°C. Thus, the values of 0.753 and 0.799 obtained for *Rana clamitans* in this study at 5° and 25°C are in agreement with these other findings. The value of 1.01 obtained at 15°C is not significant since the 95% confidence limits on this figure indicate that the actual value of n falls between 0.56 and 1.46. Closer confidence limits were not obtained at 15°C, probably because the metabolism-weight data were more variable than at 5° or 25°C.

Summary

In *Rana clamitans:*

1. Pulmonary oxygen uptake becomes increasingly important at higher temperatures.
2. Cutaneous oxygen uptake increases only slightly with increasing temperature.
3. At all temperatures, over 80% of all carbon dioxide is released through the skin.
4. Oxygen uptake is significantly affected by photoperiod only at 5°C, although 24-hour determinations might find the same effect at 15° and 25°C.
5. The relation between total oxygen uptake and weight at 5° and 25°C is the same as has been found in other frogs.

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